South African Journal of Botany 93 (2014) 137–141

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

Genetic analysis of sugar-related traits in rice grain

Y. Yang [a,b,1], Y. Rao [a,c,1], J. Xu [a,1], G. Shao [a], Y. Leng [a], L. Huang [a], L. Wang [a], L. Dai [a], G. Zhang [a], J. Hu [a], L. Zhu [a], C. Li [a], Z. Gao [a], L. Guo [a], Q. Qian [a,*], D. Zeng [a,*]

[a] State Key Lab for Rice Biology, China National Rice Research Institute, Hangzhou 310006, China
[b] Graduate School of Jiangxi Agriculture University, Nanchang, 330045, China
[c] College of Chemistry and Life Sciences, Zhejiang Normal University, Jinhua 321004, China

ARTICLE INFO

Article history:
Received 19 January 2014
Received in revised form 20 March 2014
Accepted 21 March 2014
Available online 4 May 2014

Edited by E Balazs

Keywords:
Rice (*Oryza sativa* L.)
Grain
Sugar-related traits
QTL

ABSTRACT

Sugar is the primary product of photosynthesis in plant and plays a critical role in regulating plant growth and development. In this study, quantitative trait loci (QTLs) for total soluble sugar, sucrose, and fructose contents in rice grain were identified using a double haploid population derived from a cross between *japonica* CJ06 and *indica* TN1. A total of 17 QTLs, including four QTLs for total soluble sugar content, seven QTLs for sucrose content, and six QTLs for fructose content, were detected on chromosome 1, 3, 4, 5, 6, and 8, with the LOD ranges from 2.61 to 3.85. Furthermore, among the determined varieties, we found that the total soluble sugar content in *japonica* showed higher than that in *indica*. Comparative genetic analysis showed that starch synthesis related gene is presumably involved in sugar-related metabolic activity in rice grain.

1. Introduction

Biochemical, molecular, and genetic experiments have supported a central role of sugars in the control of plant metabolism, growth, and development and have revealed interactions that integrate light, stress, and hormone signaling (Rolland et al., 2002). As the major product of photosynthesis in higher plants, sucrose is transported from leaf to grain in rice (Cho et al., 2011). The formation, transport, and storage of sucrose are necessary to support normal development of rice (Lim et al., 2006). To maintain an optimal functioning photosynthetic system, the synthesis of sucrose and starch needs to be subtly regulated (Stitt, 2004). The products can be stored as starch granules in chloroplast or transported to where they are needed by sucrose transporter, or assimilated.

The purpose of starch biosynthesis in plants is to provide a store of energy. Many plants have evolved to store large quantities of starch. For example, near 90% of the dry weight consists of starch in rice grain (Tian et al., 2009). The detail of starch biosynthesis is, however, more complicated than this – involving a number of enzymes – but can be summarized as follows. The process starts with combining of glucose-1-phosphate with adenosine triphosphate (ATP) to form adenosine diphosphate glucose (ADP-glucose), catalyzed by the enzyme AGPase. The synthesis of amylose is catalyzed by a starch granule-bound form of starch synthase (GBSS). While amylopectin is synthesized by the coordinated actions of AGPase, starch synthase (SS), starch branching enzyme (SBE), and starch debranching enzyme (DBE) (Ohdan et al., 2005). Each enzyme plays a distinct role, but presumably functions as part of a network (Tian et al., 2009). Disproportionating enzyme (DPE) and phosphorylase (PHO) are generally considered to be involved in starch degradation, but some studies suggest that both DPE and PHO may play some parts in starch biosynthesis, although the precise mechanisms of their roles are unclear (Ball and Morell, 2003; Tetlow et al., 2004).

In rice, the major storage tissues named as endosperm consist primarily of starch and a minor pool of soluble sugar which represents an alternative storage form for incoming photosynthate (Smyth and Prescott, 1989). Soluble sugar, especially the sucrose, is the primary product of photosynthesis in plant and plays a critical role for plant growth and development (Smeekens, 2000). Some rice endosperm mutations are known which increase soluble sugar content of cereal grain (Nakamura et al., 1997). The increasing soluble sugar in mutant usually results from a blockage in starch biosynthesis, and consequently, grain weight is reduced (Kubo et al., 2005). The content of soluble sugar in rice grain is minor; however, the pool of soluble sugar may affect the flavor and color reactions that take place during cooking or processing (Smyth and Henry, 1989).

QTL analysis on sugar-related traits has been carried out on some plants, such as sugarcane, tomato, and sorghum (Ming et al., 2001; Jia et al., 2010; Shiringani et al., 2010), whereas similar study on rice

* Corresponding authors at: China National Rice Research Institute, 359 Tiyuchan Road, Hangzhou, Zhejiang 310006, China. Tel.: +86 571 63370537; fax: +86 571 63370389.
E-mail addresses: qianqian188@hotmail.com (Q. Qian), dalizeng@126.com (D. Zeng).
[1] Those authors contributed equally to this work.

http://dx.doi.org/10.1016/j.sajb.2014.03.013

grain is rarely reported. Ishimaru et al. (2007) reported QTLs for sucrose, hexose and starch accumulation in the milking stage. They reckoned that *sucrosephosphate synthase 1* (*SPS1*) may play an essential role in the conversion of starch to sucrose before heading (Ishimaru et al., 2007). Nevertheless, little has been reported on the genetic base of sugar-related traits in rice grain. Here, we demonstrated the inheritance of sugar-related traits in rice grain.

2. Materials and methods

2.1. Materials

A DH population comprising 120 DH lines, which was established in our laboratory, was used in this study. This population was developed by anther culture of an F1 hybrid between the typical *japonica* CJ06 and the typical *indica* TN1 (Sogawa et al., 2004). And 35 conventional rice varieties were used to evaluate the soluble sugar content in grain. All materials were planted at the experimental farm of China National Rice Research Institute (Hangzhou, China) in 2012. Each line was planted in four rows, with six plants in each row. After harvesting, the grains of each line with three replicates were sampled for evaluation of soluble sugar, sucrose and fructose.

2.2. The evaluation of soluble sugar, sucrose and fructose

The polished grains were used to ground flour for the evaluation of sugar-related properties. The ground flour was oven-dried at 110 °C for 15 min, then stayed at 70 °C overnight. 50 mg flour was transferred into a centrifuge tube with 4 ml 80% ethanol solution. The tube was placed in 80 °C water bath with continuous stirring for 40 min. After centrifugating at 8000 *g* for 20 min, the supernatant was collected and decolorized by activated carbon. The extract was diluted with water to 10 ml for further assay.

The content of total soluble sugar was measured as described by Scott and Melvin (1953) with a slight modification. 3 ml 0.15% anthrone solution was added in a new tube carrying 0.5 ml extract, and then the tube stayed at 90 °C water bath for 15 min. The solution was used to determine the OD (optical density) value under the 620 nm wavelength. The total soluble sugar content was measured according to a standard curve, which was obtained by the concentration of a series of glucose solution against its corresponding OD value.

To evaluate the sucrose content, 0.1 ml extract was mixed with 50 μl 2 N NaOH in a tube. At the boiling water bath for 5 min, and then added 0.7 ml 30% HCl, 0.2 ml 0.1% resorcinol. The tube with the above mixture was put in 80 °C water bath for 10 min to accomplish the chromogenic reaction. The solution was used to determine the OD (optical density) value under the 480 nm wavelength. The sucrose content was measured according to a standard curve, which was generated by the concentration of a series of sucrose solution against its corresponding OD value.

To evaluate the fructose content in rice grain, 0.1 ml extract was mixed with 0.2 ml 0.1% resorcinol and 0.7 ml water in a tube under 80 °C water bath for 10 min. The solution was used to determine the OD (optical density) value under the 480 nm wavelength. The fructose content was measured according to a standard curve, which was generated by the concentration of a series of fructose solution against its corresponding OD value.

2.3. Data and QTL analysis

Population distribution and correlation analysis were performed using the SAS8.0 statistical software. A linkage map containing a total of 178 SSR and STS markers, distributed evenly on 12 chromosomes of rice, was selected to construct a rice linkage map using Mapmaker/EXP version 3.0 (Zeng et al., 2009). The map spanned approximately 1674.8 cM, with an average interval of 9.4 cM between markers. Interval QTL mapping was conducted using the software Mapmaker/QTL version 1.1 to analyze the QTLs. A likelihood of odds (LOD) threshold of 2.5 was used to declare the presence of a putative QTL in a given genomic region. The contribution to the phenotypic variance and additive effect of each QTL for relative traits were also calculated. The QTL nomenclature followed was that of McCouch et al. (1997).

Table 1
The content of rice grain total soluble sugar, sucrose and fructose in the DH population and their parents.

Traits	Parents		DH population	
	CJ06	TN1	Mean ± SD	Range
Total soluble sugar (%)	2.27 ± 0.08	1.50 ± 0.23	1.51 ± 0.31	1.04–2.54
Sucrose (%)	1.33 ± 0.07	0.94 ± 0.02	0.93 ± 0.21	0.66–1.55
Fructose (%)	0.31 ± 0.008	0.16 ± 0.004	0.19 ± 0.088	0.05–0.44

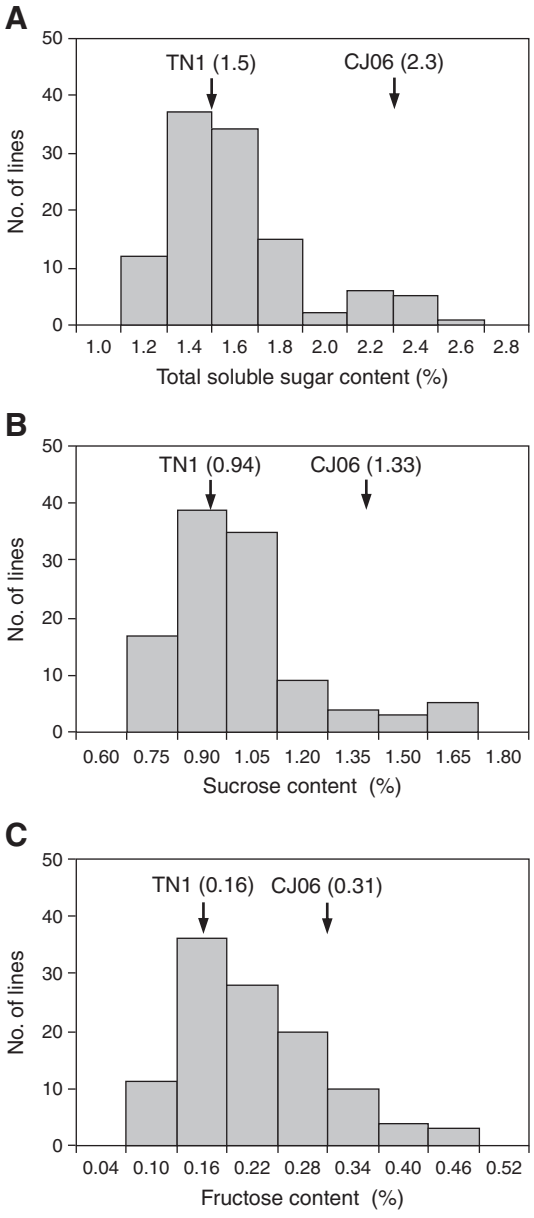

Fig. 1. The distribution of sugar-related traits in DH population. A. The total soluble sugar content in rice grain; B. THE sucrose in rice grain; C. The fructose content in rice grain.

Table 2
Correlation coefficients of sugar-related in rice grain.

	Total soluble sugar	Sucrose
Sucrose	0.4381**	
Fructose	0.5452**	0.7278**

**significant at the level of 1%.

3. Results

3.1. The content of total soluble sugar, sucrose and fructose in DH population and their parents

The content of total soluble sugar, sucrose and fructose in parents and DH population was presented in Table 1 and Fig. 1. *japonica* parent CJ06 showed higher sugar-related content than those in *indica* parent TN1. The content of total soluble sugar in CJ06 was up to 2.27%, while it is about 1.50% in TN1 (Fig. 1A). The content of sucrose in CJ06 was 1.33%, and is 0.94% in TN1 (Fig. 1B). Likewise, the content of fructose in CJ06 was twice as much as that in TN1 (Table 1, Fig. 1C). The content of total soluble sugar, sucrose and fructose showed a continuous distribution in DH population, and transgressive variation was also found in the DH population (Fig. 1A–C).

3.2. Correlation analysis of sugar content-related traits

Correlation analysis (Table 2) showed that the content of total soluble sugar exhibited a highly significant positive correlation to those of sucrose and fructose with the correlation coefficients 0.438 and 0.545, respectively. Especially, the correlation coefficient between the content of sucrose and that of fructose shows a high significance and reached up to 0.728.

3.3. QTL analysis for sugar-related traits in grain

Seventeen QTLs for sugar-related were detected in the DH population (Table 3). These QTLs were distributed on chromosomes 1, 3, 4, 5, 6, and 8 (Fig. 2). Of these, four QTLs related to total soluble sugar content were located on chromosomes 3, 5, 6, and 8, named *qSS3*, *qSS5*, *qSS6*, and *qSS8*, respectively. These QTLs had the LOD score of 2.49–3.64 and explained variation of 7.2%–15.8%. Among them, *qSS5*, the major locus for total soluble sugar content, was located in the interval of RM480–RM31 on chromosome 5, which displayed the highest LOD score with 15.8% phenotypic variation. *qSS3* and *qSS6* showed a negative additive effect, indicating that the allele from CJ06 could increase the total soluble sugar about 0.251% and 0.254%, respectively.

Seven QTLs responsible for sucrose content in rice grain were identified on chromosomes 1, 3, 4, 5, 6, and 8. The LOD score ranged from 2.62 to 3.83 with 7.6%–18.0% phenotypic variation. Among them, five QTLs including *qSu1*, *qSu3.1*, *qSu3.2*, *qSu4* and *qSu6* presented a negative additive effect; the alleles from CJ06 explained together the variance about 44.7% and increased the sucrose content about 0.551%. While the increasingly effective allele of *qSu5* and *qSu8* from TN1 can also increase the sucrose content in rice grain.

Six QTLs for fructose content were mapped on chromosomes 1, 3, 4, 5, 6, and 8, respectively, namely, *qFr1*, *qFr3*, *qFr4*, *qFr5*, *qFr6*, *qFr8*, with the LOD score of 2.39–3.42 and explained variations of 7.5%–15.0%. Among those six QTLs, *qFr4* had the highest LOD score, which was located in the interval of RM252-RM3276 on chromosome 5 and accounted for 15.0% of the phenotypic variance. Among them, *qFr1*, *qFr3*, and *qFr4* showed negative additive effects, indicating that the alleles from CJ06 can increase the fructose content in grain. On the other hand, *qFr5*, *qFr6*, and *qFr8* showed positive additive effect, indicating that these alleles from TN1 can also increase the fructose content in grain.

3.4. Comparative genetic analysis for QTLs and starch synthesis related genes

It reported that starch synthesis related genes involved in sugar metabolic activity in rice grain (Smyth and Henry, 1989; Ohdan et al., 2005; Tian et al., 2009). Thus, we further integrated the starch synthesis related genes and QTL mapping in this study. Six starch synthesis related genes, namely *AGPs2a*, *ALK*, *SPS1*, *SSIII-1*, *SSIII-2* and *SSIV-2*, were integrated with sugar-related QTLs (Fig. 2). *SPS1* is located in the internal for *qSu1* and *qFr1* between RM104 and RM1067 on chromosome 1. *SPS1* is deemed as encoding sucrose-phosphate synthase which plays a central role in the production of sucrose in photosynthetic cells and in the conversion of starch or fatty acids into sucrose in germinating seeds (Ana et al., 2000). *SSIII-1* was integrated with *qSu4* and *qFr4* between RM252 and RM3276 on chromosome 4. *SSIV-2* was co-localized with *qSS5*, *qSu5* and *qFr5* in the interval of RM480-RM31 on chromosome 5 (Dian et al., 2005; Fujita et al., 2007). We found that *ALK* was located adjacent to *qSS6* and *qSu6* on chromosome 6 (Gao et al., 2003). Besides, *AGPs2a* and *SSIII-2* associated with *qSS8*, *qSu8* and *qFr8* for the content of total soluble sugar, sucrose and fructose on chromosome 8. We further compare the sequence of those six starch synthesis related genes between two parents, five of them showed difference between CJ06 and TN1 except for *ALK* (Fig. 3). And some deletion, insertion and non-synonymous change were identified between the two parents.

Table 3
QTL identified for the content of sugar-related traits in the DH population.

Traits	Locus	Chro.	Marker interval	LOD score	Variance explained (%)	Additive effect
Total soluble sugar	*qSS3*	3	RM1350–RM3919	3.13	12.0	−0.251
	qSS5	5	RM480–RM31	2.94	15.8	0.207
	qSS6	6	RM6917–RM8258	3.64	12.6	−0.254
	qSS8	8	RM1235–RM1376	2.49	7.2	0.118
Sucrose	*qSu1*	1	RM104–RM1067	2.62	6.7	−0.095
	qSu3.1	3	RM3280–RM282	2.81	6.1	−0.094
	qSu3.2	3	RM1350–RM3919	2.96	8.1	−0.117
	qSu4	4	RM252–RM3276	3.12	14.4	−0.133
	qSu5	5	RM480–RM31	3.83	13.4	0.146
	qSu6	6	RM6917–RM8258	3.05	9.4	−0.112
	qSu8	8	RM310–RM72	3.13	9.8	0.110
Fructose	*qFr1*	1	RM104–RM1067	3.34	13.5	−0.078
	qFr3	3	RM3280–RM282	3.37	9.5	−0.069
	qFr4	4	RM252–RM3276	3.42	15.0	−0.079
	qFr5	5	RM480–RM31	3.21	13.1	0.071
	qFr6	6	RM528–RM340	2.39	7.5	0.048
	qFr8	8	RM1235–RM1376	2.42	9.5	0.054

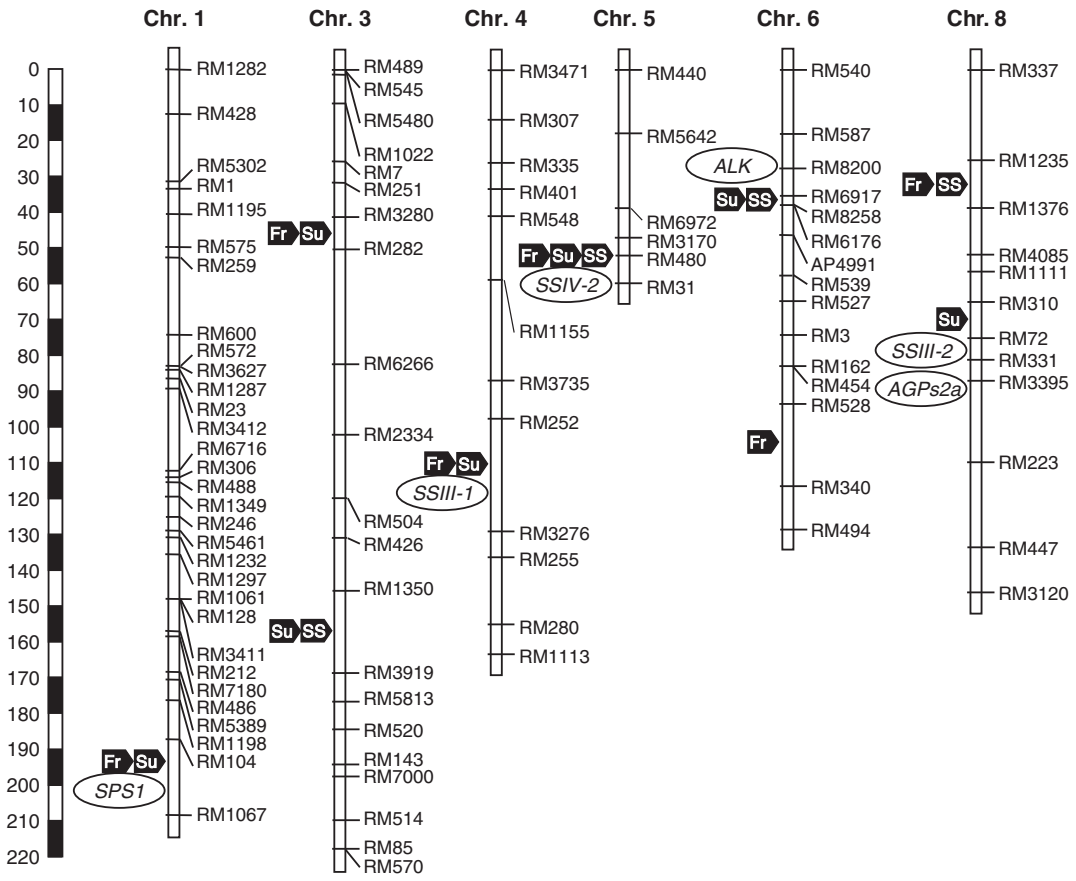

Fig. 2. QTLs for total soluble sugar content (SS), sucrose content (Su) and fructose content (Fr) in the DH population.

3.5. The comparison of soluble sugar content in different rice varieties

The sugar-related content in *japonica* variety CJ06 was higher than that in *indica* variety TN1 (Fig. 1, Table 1). To clarify whether the case is prevalent or not, we further evaluated the soluble sugar content of 35 conventional varieties including 19 *indica* rice varieties and 16 *japonica* rice varieties (Fig. 4, Table S1). Except Chunjiang 025 and Xiushui 06, the total soluble sugar content in *japonica* rice was higher than that in *indica* rice. The average content of total soluble sugar in 16 *japonica* rice is about 2.47%, while that was only 1.78% in *indica* rice variety. The results deduced that the diversity exists between the two subspecies in utilization, transportation, and conversion of soluble sugar and starch.

4. Discussion

The fate of translocated photosynthate entering the developing endosperm is to be metabolized by invertase, sucrose synthase and starch synthase etc. for starch biosynthesis. Consequently, mature cereal endosperm consists primarily of starch and a minor pool of soluble sugar (Smyth et al., 1986; Smyth and Prescott, 1989). The soluble sugar can accumulate in grain fill after starch biosynthesis (Smyth et al., 1986). This is the reason that we detected the sugar-related traits in DH population and their parents. In addition, the content of total soluble sugar, sucrose and fructose was different between CJ06 and TN1. Among the detected varieties, their total soluble sugar content ranged from 1.23% to 2.89%. We further found that the total soluble sugar content in *japonica* rice showed higher than that in *indica* rice. For example, the soluble sugar of *japonica* variety Jiahua No.1 reached up to 2.89%, which was over twice as much as that of 1.33% in *indica* variety Teqing. Smyth et al. (1986) also found the varietal difference of soluble sugar was also found in different rice varieties. When we compared the sequence of six starch synthesis related genes between CJ06 and TN1, five of them showed nonsynonymous difference.

Sucrose can be hydrolyzed *in vivo* to form fructose and glucose. The conversion among these sugars is pivotal to maintain the sugar balance in plant. It suggested that there was a tight connection between sucrose

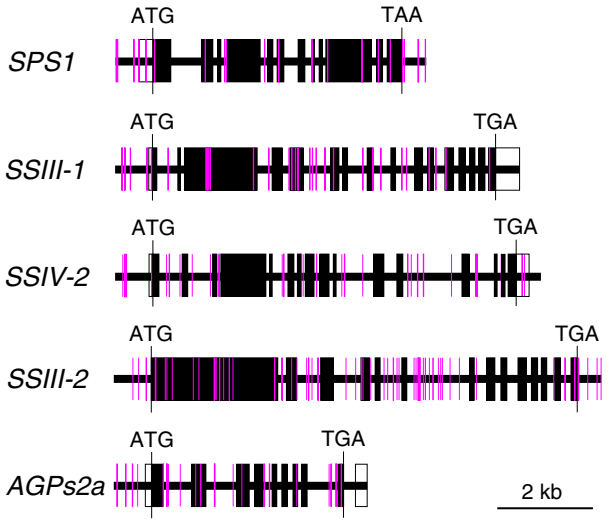

Fig. 3. The effect of starch synthesis related genes. The diversity in starch synthesis-related genes between CJ06 and TN1. Solid box and empty box indicate the exon and untranslated region, respectively; the pink lines indicate the differences between the two parents.

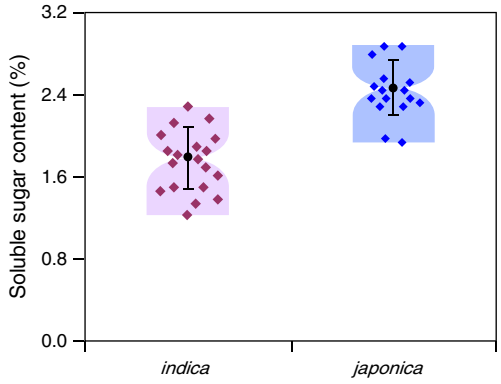

Fig. 4. The content of total soluble sugar in different rice varieties. Black dots indicate the average content of total soluble sugar in *indica* and *japonica*, respectively; the bars indicate their variations. The purple and blue dumbbell shows the distribution of total soluble sugar in *indica* and *japonica*, respectively; every diamond indicates different varieties.

and fructose, which had been indirectly proved by the correlation analysis in this study. Correlation analysis displayed the high positive correlation between the content of sucrose and fructose, which may provide clues to further understand sugar metabolism.

In this study, we identified 17 QTLs for total soluble sugar content, sucrose content and fructose content at nine intervals. Five of those intervals contained the identified starch synthesis related genes which are involved in *SPS1*, *SSIII-1*, *SSIV-2*, *SSIII-2* and *AGPase* on chromosomes 1, 4, 5 and 8, respectively. Moreover, the results showed that many sugar-related QTLs were identified in the same or adjacent interval. For example, the interval between RM480 and RM31 on chromosome 5 similarly associated with total soluble sugar content, sucrose content and fructose content. Smyth postulated that sucrose resynthesis may be involved in sucrose-phosphate synthase and sucrose phosphatase, would have to compete for precursors common to starch biosynthesis (Smyth and Henry, 1989). In addition, starch biosynthesis in plant seeds is a complex system composed of multiple subunits or isoforms (Tian et al., 2009; Wang et al., 2013).

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.sajb.2014.03.013.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31221004, 31171531), the Ministry of Agriculture of China for transgenic research (No. 2013ZX08009003-001) and the State Key Basic Research Program (2013CBA01403).

References

Ana, T.C.B., Juan, J.V.A., Miguel, M.T., Chen, L., Beatriz, X.C., William, J.L., Luis, H.E., 2000. Tissue-specific and developmental pattern of expression of the rice *sps1* gene. Plant Physiology 124, 641–653.

Ball, S.G., Morell, M.K., 2003. From bacterial glycogen to starch: understanding the biogenesis of the plant starch granule. Annual Review of Plant Biology 54, 207–233.

Cho, J.I., Kim, H.B., Kim, C.Y., Hahn, T.R., Jeon, J.S., 2011. Identification and characterization of the duplicate rice sucrose synthase genes *OsSUS5* and *OsSUS7* which are associated with the plasma membrane. Molecules and Cells 31, 553–561.

Dian, W., Jiang, H., Wu, P., 2005. Evolution and expression analysis of starch synthase III and IV in rice. Journal of Experimental Botany 56, 623–632.

Fujita, N., Yoshida, M., Kondo, T., Saito, K., Utsumi, Y., Tokunaga, T., Nishi, A., Satoh, H., Park, J.H., Jane, J.L., 2007. Characterization of SSIIIa-deficient mutants of rice: the function of SSIIIa and pleiotropic effects by SSIIIa deficiency in the rice endosperm. Plant Physiology 144, 2009–2023.

Gao, S.J., Guo, T.C., Wang, W.J., Han, J.F., 2003. Changes in the activities of enzymes involved in starch synthesis in the kernel during grain filling in winter wheat cultivars of different spike type. Scientia Agricultura Sinica 36, 1373–1377.

Ishimaru, K., Hirotsu, N., Madoka, Y., Kashiwagi, T., 2007. Quantitative trait loci for sucrose, starch, and hexose accumulation before heading in rice. Plant Physiology and Biochemistry 45, 799–804.

Jia, J.Z., Tian, L.P., Xue, L., Wei, Y.N., 2010. Dynamic QTL and correlated characters of tomato to soluble solid content. Yi Chuan 32, 1077–1083.

Kubo, A., Rahman, S., Utsumi, Y., Li, Z.Y., Mukai, Y., Yamamoto, M., Ugaki, M., Harada, K., Satoh, H., Konik, R.C., Morel, M., Nakamura, Y., 2005. Complementation of *sugary-1* phenotype in rice endosperm with the wheat *isoamylase1* gene supports a direct role for isoamylase1 in amylopectin biosynthesis. Plant Physiology 137, 43–56.

Lim, J.D., Cho, J.I., Park, Y.I., Hahn, T.R., Choi, S.B., Jeon, J.S., 2006. Sucrose transport from source to sink seeds in rice. Physiologia Plantarum 126, 572–584.

McCouch, S., Cho, Y., Yano, M., Paul, E., Blinstrub, M., Morishima, H., Kinoshita, T., 1997. Report on QTL nomenclature. Rice Genetics Newsletter 14.

Ming, R., Liu, S.C., Moore, P.H., Irvine, J.E., Paterson, A.H., 2001. QTL analysis in a complex autopolyploid: genetic control of sugar content in sugarcane. Genome Research 11, 2075–2084.

Nakamura, Y., Kubo, A., Shimamune, T., Matsuda, T., Harada, K., Satoh, H., 1997. Correlation between activities of starch debranching enzyme and a-polyglucan structure in endosperms of sugary-1 mutants of rice. Plant Journal 12, 143–153.

Ohdan, T., Francisco Jr., P.B., Sawada, T., Hirose, T., Terao, T., Satoh, H., Nakamura, Y., 2005. Expression profiling of genes involved in starch synthesis in sink and source organs of rice. Journal of Experimental Botany 56, 3229–3244.

Rolland, F., Moore, B., Jen, S., 2002. Sugar sensing and signaling in plants. The Plant Cell S185–S205.

Scott, J.T., Melvin, E.H., 1953. Determination of dextran with anthrone. Analytical Chemistry 25, 1656–1661.

Shiringani, A.L., Frisch, M., Friedt, W., 2010. Genetic mapping of QTLs for sugar-related traits in a RIL population of *Sorghum bicolor* L. Moench. Theoretical and Applied Genetics 121, 323–336.

Smeekens, S., 2000. Sugar-induced signal transduction in plants. Annual Review of Plant Physiology and Plant Molecular Biology 51, 49–81.

Smyth, D.A., Henry, E.PJ., 1989. Sugar content and activity of sucrose metabolism enzymes in milled rice grain. Plant Physiology 89, 893–896.

Smyth, D.A., Prescott, H.E., 1989. Sugar content and activity of sucrose metabolism enzymes in milled rice grain. Plant Physiology 89, 893–896.

Smyth, D.A., Repetto, B.M., Seidel, N.E., 1986. Cultivar differences in soluble sugar content of mature rice grain. Physiologia Plantarum 68, 367–374.

Sogawa, K., Sun, Z.X., Qian, Q., Zeng, D.L., 2004. Phenotypic expression of whitebacked planthopper resistance in the newly established japonica/indica doubled haploid rice population. Rice Science 11, 155–160.

Stitt, M., 2004. Metabolic regulation of photosynthesis. Photosynthesis and the Environment, pp. 151–190.

Tetlow, I.J., Wait, R., Lu, Z., Akkasaeng, R., Bowsher, C.G., Esposito, S., Kosar-Hashemi, B., Morell, M.K., Emes, M.J., 2004. Protein phosphorylation in amyloplasts regulates starch branching enzyme activity and protein–protein interactions. The Plant Cell 16, 694–708.

Tian, Z., Qian, Q., Liu, Q., Yan, M., Liu, X., Yan, C., Liu, G., Gao, Z., Tang, S., Zeng, D., Wang, Y., Yu, J., Gu, M., Li, J., 2009. Allelic diversities in rice starch biosynthesis lead to a diverse array of rice eating and cooking qualities. Proceedings of the National Academy of Sciences of the United States of America 106, 21760–21765.

Wang, J.C., Xu, H., Zhu, Y., Liu, Q.Q., Cai, X.L., 2013. OsbZIP58, a basic leucine zipper transcription factor, regulates starch biosynthesis in rice endosperm. Journal of Experimental Botany 64, 3453–3466.

Zeng, D.L., Hu, J., Dong, G.J., Liu, J., Zeng, L.J., Zhang, G.H., Guo, L.B., Zhou, Y.H., Qian, Q., 2009. Quantitative trait loci mapping of flag-leaf ligule length in rice and alignment with *ZmLG1* gene. Journal of Integrative Plant Biology 51, 360–366.